UNITED STATES PATENT AND TRADEMARK OFFICE

I, Neil Thomas SIMPKIN BA,

Deputy Managing Director of RWS Group Ltd UK Translation Division, of Europa House, Marsham Way, Gerrards Cross, Buckinghamshire, England declare;

1. That I am a citizen of the United Kingdom of Great Britain and Northern Ireland.

2. That the translator responsible for the attached translation is well acquainted with the German and English languages.

3. That the attached is, to the best of RWS Group Ltd knowledge and belief, a true translation into the English language of the accompanying copy of the specification filed with the application for a patent in Germany on 26 August 1998 under the number 198 38 802.0 and the official certificate attached thereto.

4. That I believe that all statements made herein of my own knowledge are true and that all statements made on information and belief are true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent application in the United States of America or any patent issuing thereon.

For and on behalf of RWS Group Ltd

The 18th day of May 2009

FEDERAL REPUBLIC OF GERMANY

Certificate

Boehringer Mannheim GmbH

of

Mannheim/Germany

have filed a Patent Application under the title:

"Improving binding assays through multiepitope analysis and combination of antigen and antibody determination"

on 26 August 1998 at the German Patent and Trademark Office, and declare that they claim, for this application, the inner priority of the Application made in the Federal Republic of Germany on 22 June 1998, File No. 198 27 714.8.

The company name of the applicant has been changed to: Roche Diagnostics GmbH.

The attached document is a correct and accurate reproduction of the original submission for this Patent Application.

The German Patent and Trademark Office has for the time being given the Application the symbol G 01 N 33/543 of the International Patent Classification.

Munich, 22 April 1999

President of the German Patent Office

pp

Faust

File No: 198 38 802.0

Improving binding assays through multiepitope analysis and combination of antigen and antibody determination

Description

The invention relates to a method for detecting one or more analytes in a sample, where the analyte is detected using different reagents capable of binding to the analyte. The invention further includes a solid phase for detecting an analyte, where the solid phase includes a nonporous support and spatially separate assay areas, where each assay area contains different reagents. The invention also relates to a method for the simultaneous determination of an antigen and of an antibody specifically directed against this antigen, and to a solid phase for carrying out this method.

A large number of analytes can be determined by immunological detection methods. Such immunological detection methods make use of the specific binding ability of analytes with particular reagents, such as, for example, antigen-antibody interactions. It is possible in principle to carry out immunological determinations in a number of assay formats, such as, for instance, the sandwich assay format, the indirect assay format, the back-titration format or the bridge format.

A reliable detection of infectious diseases, e.g. of an infection with viruses such as, for instance, HIV, HBV or HCV, is of particular interest in order to be able to diagnose the disorder as early as possible in the people affected. In general, immunological determinations of antibodies against HIV, HBV or HCV are carried out in an indirect assay format or by means of a bridge format. To detect the antibodies, in this case usually mixtures of various proteins or peptides which include epitopes from the core and envelope regions of the pathogen are used. This mixture is immobilized on a

support, i.e. a solid phase. Since the classification as HIV-positive is of great importance for the individual and false-positive results may have disastrous consequences, it is currently necessary to check, in a confirmation assay, all the positive results obtained with this immunological determination in routine assays. Western blotting is normally used as confirmation assay and entails the individual protein constituents of a viral lysate being blotted onto a porous support. In the case of HCV, however, it is very difficult to culture the virus system. For this reason, the confirmation assay carried out in this case is not a Western blotting with viral lysate but an RIBA (recombinant immunoblot assay) which is an immuno-dot blot which includes recombinant proteins or peptides as assay reagents.

A great disadvantage of the currently used routine assays is that mixtures of 5 to 10 or more antigens, depending on the analyte, are employed for the detection. Although there are continual improvements in the routine assays, it has not yet been possible to achieve a complete abandonment of confirmation assays. For example, in the Enzymun® HIV assay (Boehringer Mannheim), a mixture of about 5 different antigens is used, and they are both biotinylated and digoxigenin-labeled for the detection. Although the assay functions well, the use of antigen mixtures with such a large number of different antigens means that the individual antigens immobilized or bound on the solid phase can no longer be present in a concentration which is optimal for detection. The binding capacity of the solid phase with such a mixture of a large number of components is no longer sufficient to bind all the antigens in the optimal concentration. In addition, the use of an antigen mixture for coating an assay surface results in the various antigens competing for the binding sites on the solid phase, and the different size ratios leading to different diffusion rates and to different steric

effects. In the case of direct coating, for example, hydrophobic antigens are preferentially bound to the plastic surface, while at the same time more hydrophilic antigens are displaced. This leads to on the one hand only poorly reproducible results being obtained, and on the other hand to the concentration of particular antigenic epitopes being so low that a significant detection is no longer possible.

A further disadvantage of the use of antigen mixtures in routine assays is that the risk of an increased nonspecific binding is distinctly increased through the mixture of different antigens, in turn leading to an increase in false-positive results. The effect of this is that the cutoff limit must be set at a relatively high level in the routine assays used to date, and thus sensitivity is lost. Especially in Western blotting, the number of false-positive results increases distinctly because of nonspecific binding, owing to the foreign proteins present in the viral lysate, so that at least 2 reactive bands are required for a positive result.

Attempts have been made to improve the sensitivity of these detection methods further. EP 0 461 462 A1 describes an immunoassay for detecting viral antibodies with the aid of an indirect assay design. In the immuno-dot blot described in EP 0 461 462 A1, instead of a usual viral lysate, purified recombinant proteins are applied individually in discrete assay areas on a porous support, resulting in an assay format which, owing to the use of purified proteins, is more sensitive than a Western blot.

EP 0 627 625 A1 relates to a method for detecting viral antibodies in a sample by means of a bridge design. This method is also an RIBA (recombinant immunoblot assay) in which a plurality of antigens are applied spatially separately to a solid phase composed of a

porous material, reference being made to the necessity to use a solid phase composed of porous material.

EP 0 445 423 A2 relates to a method for detecting HCV antibodies with the aid of a plurality of epitopes of an HCV antigen. EP 0 445 423 A2 also describes an immuno-dot assay for antibody determination, with a higher sensitivity being achieved through the use of particular, improved antigens.

However, in these methods described in the prior art, owing to the use of a porous support the defined application of a predetermined amount of reagent is difficult. In particular, there is the risk that the individual assay spots applied will become intermingled. These disadvantages become more serious as the size of the applied spots decreases, so that these methods are unsuitable in particular for miniaturized assay systems. In addition, the manipulation of paper strips is difficult to automate and thus inconceivable as routine assay.

One object of the invention was therefore to provide a method by which the disadvantages occurring in the prior art can be at least partly eliminated.

This object is achieved according to the invention by a method for detecting an analyte in a sample, comprising the steps:

(a) providing a solid phase which includes a nonporous support and at least two spatially separate assay areas, where the assay areas each comprise different, immobilized analyte-specific receptors,

(b) contacting the sample with the solid phase and at least one free analyte-specific receptor which carries a signal-emitting group or is capable of binding to a signal-emitting group, and

(c) detecting the presence or/and the amount of the

analyte by determining the signal-emitting group on the assay areas.

The immobilized analyte-specific receptor may be bound either directly or indirectly via one or more receptors to the solid phase. The binding can take place for example by adsorptive or covalent interactions, but preferably by specific high-affinity interactions, e.g. streptavidin or avidin/biotin or antibody-antigen interactions.

The free analyte-specific receptor may itself carry a signal-emitting group or be capable of binding to a signal-emitting group. In the latter case, the detection reagent consists of a plurality of components.

The analyte may be a homogeneous or a heterogeneous population, e.g. a heterogeneous antibody population, an antigen mixture or a mixture of, where appropriate, different antigens and antibodies, with the antigens and antibodies being respectively derived from and induced by one or more pathogens. In the case of heterogeneous analyte populations, the individual assay areas bind a subpopulation of the analyte to be determined. The analyte-specific receptors which are immobilized in each case on an assay area are different, i.e. they bind according to the invention preferably to different epitopes of a homogeneous analyte such as, for instance, of an antigen, to different analyte subtypes such as, for instance, antigen subtypes or/and to different analyte types such as, for instance, different antigens or/and antibodies.

It has surprisingly been found that the sensitivity of detection assays such as, for instance, antibody assays can be distinctly improved through the use of panel assays in which the various reagents, e.g. various antigens, are applied as single spots, i.e. singly to

separate assay areas. It is possible through the multiepitope analysis according to the invention, i.e. the simultaneous separate detection of a plurality of subpopulations of an analyte or pathogen, such as, for instance, HIV, for the sensitivity and reliability of detection assays to be considerably increased.

If a positive assay result is obtained on one or more, in some cases on at least two, assay areas, this is assessed as the presence of the analyte in the sample.

It is possible through the use according to the invention of a nonporous support to apply the reagents in defined areas. This is important in particular with miniaturized assay formats. Correspondingly, the assay areas preferably have a diameter of from 0.01 to 1 mm, more preferably from 0.1 to 0.5 mm and most preferably from 0.1 to 0.2 mm.

Solid phases with a plurality of assay areas, which are also referred to as array systems, are preferably used. Such array systems are described for example in Ekins and Chu (Clin. Chem. 37 (1995) 1955-1967) and in US patents 5 432 099, 5 516 635 and 5 126 276.

The solid phase used according to the invention includes a nonporous support which can be used for detection methods. The nonporous support may in this case consist of any nonporous material. The support preferably includes a plastic, glass, metal or metal oxide surface. The support particularly preferably has a polystyrene surface. Spatially discrete regions (assay areas) are arranged on this support. Reagents such as, for instance, immobilized solid-phase receptors are applied to these assay areas. The reagents are immobilized on the assay areas by known methods, e.g. by direct adsorptive binding, by covalent coupling or by coupling via high-affinity binding pairs, e.g. streptavidin/biotin, antigen/antibody or

sugar/lectin.

It is particularly advantageous for the spatially separate assay areas to be separately loaded with different reagents. It is possible through the individual application of the different assay areas to choose for each reagent, for example for each individual antigen, the optimal solid-phase concentration and the optimal coating conditions, i.e. in the form of specific buffer receptors. This makes it possible for each individual analyte-specific receptor, e.g. each individual antigen, to be coated up to the maximum binding capacity of the area, whereas with the assays disclosed to date it was possible for each receptor, e.g. each antigen, to be bound only for part of the available binding capacity. In addition, through the separate application of the different reagents, no competition of the individual reagents, for example of the antigens, for the binding sites on the solid phase takes place. Correspondingly, it is preferred for in each case only one reagent which is capable of specific binding with the analyte to be determined to be bound on each assay area, so that each assay area contains only a single type of an immobilized analyte-specific receptor. This reagent can where appropriate be diluted by inert diluent molecules in order to form an optimal homogeneous binding phase. Inert diluent molecules are molecules which bind to the solid phase but do not enter into any interaction with the analyte or other sample constituents. Suitable diluent molecules are described for example in WO 92/10757 and in EP 0 664 452 A2.

It has been found with assay areas on which only a single reagent capable of binding to the analyte, such as, for instance, an antigen, is bound that the nonspecific binding is distinctly reduced. Thus, for example, no measurable nonspecific binding is to be observed on application of different antigens as single

spots, whereas an assay spot onto which a mixture of a plurality of antigens has been applied shows a distinctly measurable nonspecific binding.

The analyte is detected in the method of the invention in a known manner by using suitable labeling groups, e.g. fluorescent labeling groups, chemiluminescent groups, radioactive labels, enzyme labels, colored labels and sol particles. An alternative possibility with suitable solid phases is to detect the interaction of constituents of the detection medium with the assay areas also by determining the layer thickness of the respective areas, e.g. by plasma resonance spectroscopy.

The defined assay areas may moreover comprise, to distinguish from inert regions of the solid phase, a detectable and analyte-nonspecific labeling group which can be detected in addition to the analyte-specific coating group and does not interfere with it. One example of such an analyte-nonspecific labeling group is a fluorescent labeling group which fluoresces at a wavelength which is different from the fluorescence wavelength of an analyte-specific labeling group. The analyte-nonspecific labeling group is preferably immobilized, just like the solid-phase receptor, via a high-affinity binding pair, e.g. streptavidin/biotin.

A further increase in sensitivity can be achieved through the use of a universal detection reagent. It is possible beforehand to employ for each analyte molecule a separate detection reagent which binds to the analyte molecule and carries a label such as, for instance, an enzyme, a fluorescent marker or fluorescent latex particles. However, the combination of a plurality of labeled detection reagents often means that the concentration of labels is very high, so that the nonspecific binding naturally increases greatly. This problem can be solved through the use of a universal

detection reagent. Fluorescence-labeled latex particles are preferably used according to the invention as universal detection reagent. In this case, an analyte-specific first receptor which does not itself carry a signal-emitting group is used for the specific binding of the analyte molecule. A universal second labeled receptor, i.e. a receptor which binds analyte-independently to a plurality, preferably to all, of the first receptors used, binds to this analyte-specific first receptor. The coupling of the second receptor to the labeling group can take place by adsorption, covalently via functional groups or take place via high-affinity binding pairs, e.g. streptavidin/biotin, antigen/antibody or sugar/lectin. The well-known dig/anti-dig system is preferably used.

A further disadvantage of the bridge assays which are carried out for example as 1-step reaction is that the solid-phase receptor (e.g. biotinylated HIV gp41) and the free detection receptor (e.g. digoxigenylated HIV gp41) must be supplied in the ratio of 1:1 in order to achieve an optimal signal. This is disadvantageous because, owing to the limited binding capacity of the solid phase, the concentration of the individual solid-phase receptors is often suboptimal and thus cannot be favorable for the detection receptor either.

It is possible with the method of the invention for the solid-phase receptors already to be bound to the solid phase in optimal concentration. It is also possible in addition for the detection receptor to be supplied in optimal concentration, because receptor conjugates with digoxigenin or biotin are, in contrast to enzyme-labeled receptors, prone to nonspecific binding to only an insubstantial extent. These reagents can be employed in excess, so that imprecision in the addition of the receptor does not impinge on the assay precision.

It is possible by specific binding of the analyte to be

determined onto the reagent immobilized on the assay area, e.g. a solid-phase receptor, for the presence or/and the amount of the analyte in a sample to be determined. It is possible by combined evaluation of the different assay areas which each contain different reagents capable of specific binding to the analyte for the sensitivity of the detection method to be markedly improved in particular through a reduction in false-positive results and the unambiguous identification of correct-positive results. The method of the invention is of particular interest for picking up and eliminating nonspecific bindings in qualitative assays with high requirements for specificity, such as, for instance, in assays for infections (e.g. HIV).

It is possible through the use according to the invention of arrays, i.e. solid phases which include at least two, more preferably at least three, most preferably at least five, and up to one thousand, more preferably up to one hundred, spatially separate assay areas to configure at least one of these assay areas in such a way that it represents a control area. Consequently, the method of the invention preferably includes the use of a solid phase which additionally includes at least one, more preferably two and most preferably at least five control areas. The integration of control spots into the solid phase makes it possible easily and rapidly to identify incorrect results resulting from interference. Besides the specific assay areas it is additionally possible to measure a sample-specific background and thus define a sample-specific cutoff. The use of an assay array and the use of control spots makes it possible to reduce the cutoff limit. The cutoff index is a limit employed in assay methods in order to be able to distinguish between positive and negative values. Such a cutoff index is important especially in assay methods which relate to infectious diseases. It is possible with the aid of the method of the invention to make a positive/negative

differentiation with a considerably smaller probability of error.

On use of a plurality of assay areas which are in each case provided for determining different analyte molecules it has often proved suitable to define the cutoff index assay area-specifically in order to obtain an increased assay specificity (i.e. correct differentiation between positive and negative values) while the sensitivity remains the same.

The method of the invention can be employed for any detection methods, e.g. for immunoassays, nucleic acid hybridization assays, sugar-lectin assays and similar methods. The method of the invention is also suitable in principle for detecting any analytes in a sample. The analyte is particularly preferably detected via specific interactions with one or more reagents capable of binding to the analyte, i.e. receptors which are preferably selected from proteins, peptides, antibodies, antigens, haptens and nucleic acids.

Whereas an essential advantage of the method of the invention in the first place is to improve the sensitivity of the detection a single analyte, it is also possible with a suitable choice of the assay areas to determine a plurality of analytes simultaneously with high sensitivity.

The present invention further relates to a solid phase for detecting an analyte in a sample, which is characterized in that it includes a nonporous support and at least two spatially separate assay areas, where the assay areas each comprise different reagents which are capable of specific binding with the analyte to be determined.

The assay areas preferably comprise in each case different reagents which bind to different epitopes

or/and subtypes of an analyte or/and to different analyte types.

Miniaturized assay formats are preferably used in order to accommodate a maximum number of assay areas on a solid phase. The distance between the individual assay areas is chosen so that intermingling of the applied reagents is impossible. It is normally sufficient for this purpose for the edges of the assay areas to be at a distance of from 0.05 to 5 mm. Between the assay areas there is preferably an inert surface which is capable of binding neither with the analyte nor with any other constituents of the sample.

The solid phase of the invention can be employed in any detection methods, e.g. in immunoassays, nucleic acid hybridization assays, sugar-lectin assays and the like. It is preferably used in an immunoassay for detecting antibodies or/and antigens.

The invention further encompasses an assay kit for detecting an analyte in a sample, which includes a solid phase of the invention, and labeled detection reagents. Labeled detection reagents are known to a person skilled in the art and generally include a labeling group and a group which is capable of specific binding and which makes detection of the analyte possible. Suitable labeling groups are for example fluorescent, chemiluminescent, enzyme, radioactive or particle (sol) labeling groups. The group which is capable of specific binding may, for example, be capable of binding with the analyte complex formed or, in the case of competitive assay formats, with other constituents of the detection system. The assay kit preferably includes a universal conjugate as detection reagent, in particular fluorescent-labeled latex particles, which is capable of binding with the detection receptors specific for the analyte.

A further problem with conventional routine assays is that the simultaneous determination of an antigen and of an antibody specifically directed against this antigen cannot be carried out in one measurement. For this reason, for example, in so-called HIV combination assays a determination of the antigen p24 and of antibodies against other HIV antigens is carried out simultaneously. With such an assay it is then possible only to determine antibodies against other HIV antigens such as, for example, gp41 or gp120, whereas determination of antibodies against p24 is not possible.

US 5 627 026 describes a method for detecting an antibody and an antigen in a biological sample. Thus, for example, an assay for determining the FeLV antigen and the FIV antibody is described. In the method of US 5 627 026 it is also possible when determining an antigen only to detect antibodies directed against other antigens in the same assay.

A further object of the present invention is therefore to provide a method for the simultaneous determination of an antigen and of an antibody specifically directed against this antigen in a sample. This object is achieved by a method including the steps

(a) providing a solid phase on which an immobilized receptor capable of binding with the antigen to be determined is applied in a first assay area, and an immobilized receptor capable of binding with the antibody to be determined is applied in a second assay area spatially separate therefrom,

(b) contacting the sample with the solid phase and with a free analyte-specific receptor which carries a signal-emitting group or is capable of binding with a signal-emitting group, and

(c) detecting the presence or/and the amount of the antigen and of the antibody by determining the signal-emitting group on the solid phase.

The antigen is detected preferably by using a sandwich assay and the antibody is detected preferably by using a bridge design, a back-titration design or an indirect assay format.

The antibody is preferably detected by using a back titration. The advantage of this is that on simultaneous use of a sandwich assay for detecting the antigen it is impossible for detection molecules to influence one another, because in this case the same detection reagent can be used for detecting the antigen and detecting the antibody. For example, for a sandwich assay for detecting an antigen, e.g. HIV p24, an antibody directed against this antigen is immobilized on an assay area. It is possible then to use as free receptor which serves for detection a second, directly or indirectly labeled antibody directed against the antigen, e.g. a digoxigenylated anti-p24 antibody. To detect the corresponding antibody directed against the antigen, e.g. an anti-p24 antibody, using a back titration, an antigen capable of binding with the antibody, e.g. p24, or a fragment thereof, is immobilized on a further assay area. The detection reagent used is likewise the second labeled antibody directed against the antigen, e.g. a digoxigenylated anti-p24 antibody which competes with the analyte, which is for example the natural anti-p24 antibody present in the sample, for binding to the immobilized antigen. It is thus possible for the preferred simultaneous detection of a p24 antigen and of the anti-p24 antibody directed against it in each case to use the same detection reagent, for example a digoxigenylated anti-p24 antibody.

If the antigen is detected by a sandwich assay, and the antibody is detected in parallel by a bridge assay or an indirect assay it is necessary to use specific assay reagents in order to preclude mutual influencing of the

detection reagents. In an indirect assay for detecting an antibody, e.g. an anti-p24 antibody, for example an antigen which is specific for the antibody to be detected, e.g. p24, is immobilized on an assay area. The antibody is then detected by using a labeled antibody which, although recognizing the antibody to be detected, does not recognize the immobilized antigen, for example a digoxigenylated anti-human IgG antibody. It is possible in the parallel antigen determination in sandwich format to employ on the detection side one or more antibodies, preferably monoclonal antibodies, whose epitope binding sites are known. It is possible at the same time in the antibody determination in an indirect assay format or in a bridge format not to use any native or recombinant antigens which comprise epitopes capable of binding with the detection antibody, because otherwise an unwanted reaction takes place. It is necessary instead to use predetermined recombinant antigens or peptide antigens without these epitope binding sites to which the detection antibody (antibodies) employed are not capable of binding.

It is possible by the multiepitope analysis according to the invention with array systems to carry out a combination of antigen and antibody detections for a particular antigen and an antibody directed against this particular antigen. This procedure makes it possible to close the diagnostic gap, which exists with the methods known in the prior art, between the first appearance of an antigen and the appearance of antibodies at a different time, and to categorize a sample as positive or negative very early. Samples are normally taken from patients, with the sensitivity of an assay being determined by identification of positive samples as early as possible. In the event of an infection, the various markers indicating these infections, such as, for example, antigens or antibodies directed against these antigens, appear with a different time course.

The multiepitope method of the invention with an array arrangement additionally makes it possible, through the spatially separate arrangement of the individual assay areas, for antigen and antibody assays to be specifically differentiated. The advantage of the method of the invention is evident in particular with HIV assays. A preferred example of the method of the invention is the simultaneous detection of an HIV antigen and antibodies directed against it, e.g. the p24 antigen and the corresponding anti-p24 antibody. In the event of an HIV infection, p24 antigens appear first. They can be detected with an antigen assay, but not with an antibody assay. The appearance of the antigens is followed by formation of antibodies against these antigens in the body. However, it is not possible with conventional combination assays to combine the p24 antigen assay with an anti-p24 antibody assay; on the contrary the p24 antigen assay is combined with an anti-gp41 antibody assay. Since the formation of anti-gp41 antibodies may, however, take place at a time subsequent to the formation of anti-p24 antibodies, false-negative results may be obtained with conventional methods in the period until anti-gp41 antibodies are formed. The method of the invention is by contrast more reliable because anti-p24 antibodies can also be determined.

It is preferred according to the invention for the coating, which is capable of binding, of the first assay area in which the antigen is to be detected to be formed from immobilized antibodies which are specific for epitopes of the antigen to be detected. It is possible, owing to the array structure which is preferably used, for a plurality of antibodies which are specific for different subtypes of the antigen to be detected to be applied in separate assay areas. The antibodies are selected appropriately for the antigen to be analyzed. In screening for a viral infection,

preferably anti-HIV-I antibodies, anti-HIV-II antibodies, anti-HBV antibodies or/and anti-HCV antibodies are assayed. Analogously, the coating, which is capable of binding, of the further assay areas on which an antibody is to be detected preferably includes antigens which are specific for the antibody to be detected. It is also possible in principle in this case to use any antigens appropriate for the particular assay, and preferably antigens or epitopes thereof from HIV-I, HIV-II, HBV or/and HCV are used.

It is possible through the use of a nonporous solid phase to obtain particularly good results with the method of the invention. A nonporous solid phase has advantages in particular in the application of the assay reagents, with a defined application without intermingling of the individual assay areas being possible. It is further possible on use of nonporous assay phases to miniaturize the assay format. It is possible with miniaturized assay formats to apply a large number of assay areas to a single nonporous solid phase.

Detection of the binding of an antigen or antibody to the assay areas is preferably carried out by using labeled antibodies directed against the analyte. In the case of detection of the antigen in a sandwich format, a labeled antibody directed against this antigen is used. The same labeled antibody also serves to detect the analyte antibody in the competitive format, e.g. a back titration. It is thus possible, by spatially separate evaluation of the individual assay areas, to detect with a single detection reagent both antigen and the antibody specific for this antigen, without mutual impairment of the two detection methods. Suitable labeling substances for labeling antibodies are known to a person skilled in the art and include, for example, fluorescent groups, chemiluminescent groups, radioactive labels, enzyme labels, colored labels and

sol particles. It is preferred to use a universal detection reagent, in particular fluorescence-labeled latex particles, which is capable of binding for example with the detection receptors.

Particularly good results are obtained with the method of the invention when the coatings capable of specific binding are applied separately to the individual assay areas. This makes it possible to utilize the binding capacity of the individual assay areas optimally and to produce coatings capable of optimal binding. It is possible where appropriate for the reagents capable of binding to be diluted by diluent molecules in order to improve further the binding ability of the coating. Suitable diluent molecules are molecules which do not bind with the analyte to be determined and which also show no nonspecific interaction or binding with other constituents of the sample, possibly leading to false-positive results (cf. WO92/10757, EP 0 664 452 A2). The coating in the individual assay areas is particularly preferably formed in each case from a single molecule type capable of specific binding. In this case, different reagents capable of binding with the analyte are applied in different test spots. It is possible in this way to increase further the sensitivity of the method of the invention.

The present invention further relates to a solid phase for simultaneous determination of an antigen and of an antibody specifically directed against this antigen, comprising at least a first assay area and at least a second assay area, which is characterized in that the first assay area includes a coating capable of specific binding with an antigen, and the second assay area includes a coating capable of specific binding with an antibody directed against the antigen, the coatings being homogeneous and each comprising only a single type of a reagent capable of binding. The coatings are uniformly applied to the assay areas, meaning that they

are homogeneous. Besides the reagent capable of binding, the assay areas may include inert diluent molecules which are not able to enter into interactions either with the analyte to be detected or with other constituents of the sample.

Whereas it is possible in principle to use any support materials, the assay areas of the solid phase of the invention are preferably applied to a nonporous support. Through the use of nonporous surfaces is in particular a miniaturization of the assay format and simultaneous determination of a large number of assay areas.

The solid phase of the invention is particularly suitable for use in an immunoassay for simultaneous detection of an antigen and of an antibody specifically directed against this antigen. It is possible in this way to improve further the sensitivity and reliability of immunoassays.

The invention also includes a test kit for simultaneous determination of an antigen and of an antibody specifically directed against this antigen, which includes the solid phase of the invention, and labeled detection reagents for detecting antigen and antibody bound to the assay areas. Suitable detection reagents are for example labeled antibodies, it being possible to select the label from the abovementioned groups.

A further problem with the route assays available to date is that all the antigens and antibodies necessary for an assay, for example an HIV assay, are mixed, and a cutoff limit which is optimal for this mixture is established for the detection method. However, through the use of a common cutoff limit for all parameters, the cutoff limit is determined and limited by the nonspecific binding of the worst starting material. The present invention therefore further relates to a method

for detecting an analyte in a sample, including the steps:

(a) providing a solid phase which includes a support and at least two spatially separate assay areas, where the assay areas each comprise different, immobilized analyte-specific receptors,

(b) contacting the sample with the solid phase and at least one free analyte-specific receptor which carries a signal-emitting group or is capable of binding to a signal-emitting group, and

(c) detecting the presence or/and the amount of the analyte by determining the signal-emitting group on the assay areas, where a signal above a predetermined assay area-specific limit is classified as positive and below a predetermined assay area-specific limit is classified as negative.

It is possible through the use of predetermined assay area-specific limits for each individual assay area to improve markedly the specificity of detection methods, while the sensitivity remains at an unchanged high level. The limit or cutoff index is determined by means of the variables signal of the sample, background of the sample and background of a negative control. The cutoff index (COI) is normally calculated for example according to the formula:

$$COI = signal_{sample} - background_{sample} / n \times background_{negative\ control}$$

A usual value for n is for example 2. The factor n - and thus the cutoff index - can be increased for particular assay areas where false-positive samples are observed, it being possible for n to be a number between 2 and 100, preferably between 2 and 10.

The limits are preferably determined in each case individually for an assay area. This means that

different limits or cutoff indices are established for the different assay areas; in particular, the limits are established differently for at least two assay areas. Preferred embodiments of this method make use of the features described above.

The invention is explained further by the following examples.

Examples

1. Assay for anti-HIV antibodies with a plurality of antigen-specific assay areas by means of microspot technology

Microspot is a miniaturized ultrasensitive technology ideally suitable for simultaneous determination of various parameters in a single measurement operation. In the case of determination of anti-HIV antibodies, various HIV detection antigens are applied in each case singly by means of an inkjet method onto an assay area (spot) in so-called arrays on a polystyrene support. When the assay is carried out, 30 µl of sample diluted in the ratio 1:1 with sample buffer are pipetted onto the support provided with assay areas and incubated at room temperature while shaking for 20 minutes. Aspiration of the sample and washing with washing buffer are followed by addition of 30 µl of reagent solution 1, which comprises a mixture of all digoxigenin-labeled HIV antigens, and incubation at room temperature with shaking again for 20 minutes. Aspiration of reagent solution 1 and washing with washing buffer are followed by addition of 30 µl of reagent solution 2 with detection reagent. Fluorescent latex particles which are 100 nm in size and are covalently coated with an anti-digoxigenin antibody serve as universal detection reagent.

This detection reagent is again incubated at room

temperature with shaking for 20 minutes, subsequently aspirated, washed and sucked dry. The assay areas are then irradiated with an He-Ne laser with a wavelength of 633 nm, and the fluorescence is measured at a wavelength of 670 nm with a CCD camera.

The solid phase comprises specific assay areas with the following immobilized antigens:

- recombinant p24 polypeptide
- recombinant reverse transcriptase (RT)
- gp41 peptide 1
- gp41 peptide 2

The sample buffer used was a 50 mM tris buffer of pH 7.6 with the following additions: 0.05% Tween 20, 0.5% bovine serum albumin (BSA), 0.1% bovine IgG, 0.01% methylisothiazolone, 3% peptone.

Reagent solution 1 used was the sample buffer described above comprising the following assay-specific antigens:

- digoxigenin-labeled recombinant p24
- digoxigenin-labeled recombinant reverse transcriptase
- digoxigenin-labeled gp41 peptide 1
- digoxigenin-labeled gp41 peptide 2

Reagent solution 2 used was a 50 mM tris buffer of pH 8.0 with the following additions: 0.05% Tween 20, 0.9% NaCl, 0.5% BSA, 0.1% Na azide and 0.01% of fluorescence-labeled latex particles coated with a monoclonal anti-digoxigenin antibody.

2. Comparison of an anti-HIV antibody assay in microspot format with conventional methods

In this experiment, so-called seroconversion samples were measured. These samples are taken at consecutive times from various people whose serum findings are

converted from HIV-negative to HIV-positive. A more sensitive test method means earlier detectability of an HIV-specific antibody signal. The samples are measured with the method of the invention (microspot) and for comparison with a known method (Enzymun® from Boehringer Mannheim). The HIV-specific starting materials used in this case were identical in both assay systems, which therefore differ in particular only through the separate single spot analysis. The cutoff indices (cutoff index = $signal_{sample}$-$signal_{background}$/ 2×$signal_{negative\ control}$) of the two methods are entered in the following table and additionally compared with Western blot data:

Seroconversion panel from BBI[1]		Day of sampling	p24	RT	gp41 peptide 1	gp41 peptide 2	Western blot	Enzymun®
R	2nd sampling	2	0.0	0.0	0.0	**1.3**	negative	0.5
	3rd sampling	7	22.2	0.0	0.0	2.4	neutral	15.4
	4th sampling	13	17.4	2.6	4.4	36.4	positive	36.0
AB	1st sampling	0	0.0	0.0	0.6	0.0	negative	0.3
	2nd sampling	28	0.0	0.0	0.4	**1.1**	negative	0.7
	3rd sampling	33	0.0	0.0	5.5	54.4	negative	24.2
	4th sampling	35	0.8	0.2	6.0	33.2	positive	26.7
	5th sampling	37	15.2	5.1	4.6	33.0	positive	28.9
AD	5th sampling	21	0.0	0.0	0.0	0.0	negative	0.3
	6th sampling	25	0.2	0.0	**1.6**	**3.7**	positive	0.9
	7th sampling	28	14.1	0.3	11.1	65.5	positive	24.5
AG	3rd sampling	13	0.0	0.0	0.0	0.0	negative	0.4
	4th sampling	27	0.0	0.0	0.0	**1.1**	negative	0.6
	5th sampling	34	6.0	5.3	0.0	106.9	positive	8.1
	6th sampling	50	6.1	5.4	0.0	65.4	positive	3.1
	7th sampling	78	1.0	6.8	0.0	23.9	positive	1.6
	8th sampling	163	**1.5**	**5.3**	0.0	**4.9**	positive	0.6
	9th sampling	194	**2.3**	**2.5**	0.0	**2.8**	positive	0.7
AI	1st sampling	0	0.0	0.0	**1.2**	0.1	neutral	0.8
	2nd sampling	7	0.6	0.5	54.7	44.9	positive	30.2
	3rd sampling	11	1.1	0.7	18.8	22.5	positive	30.2

* Boston Biomedica Inc.

This comparison shows that it was possible markedly to improve the sensitivity by comparison with known assays through the division into single spots each having optimal antigen concentrations. 7 samplings from the 5 seroconversion panels are identified as positive earlier. This corresponds to an earlier detection of HIV infection of from 3 to 7 days, depending on the panel. Also by comparison with the Western blot, a marked increase in the sensitivity was achieved, with 6 samplings identified earlier.

3. Comparison of a combined determination of HIV p24 antigen and anti-gp41 and anti-RT antibodies in microspot format with conventional methods

To evaluate the sensitivity, so-called seroconversion samples were again measured. The determination took place with the method of the invention (microspot), and the data obtained therein were compared with the best anti-HIV assay currently available (see datasheets of the producers of seroconversion panels, e.g. BBI) and with the Enzymun® combination assay from Boehringer Mannheim (combined determination of p24 antigen and anti-HIV antibodies).

The following assay areas (single spots) (produced as in Example 1) were used for the microspot assay format:

- monoclonal anti-p24 antibody A for determining the p24 antigen of HIV subtype B
- monoclonal anti-p24 antibody B for determining the p24 antigen of HIV subtype B and O
- gp41 peptide 1 for determining antibodies against gp41
- gp41 peptide 2 for determining antibodies against gp41
- recombinant reverse transcriptase (RT) for

determining antibodies against RT

The HIV-specific starting materials used in the microspot assay were comparable with the starting materials used in the Enzymun® assay, so that the microspot assay differs from the Enzymun® method in particular only through the separate single spot analysis. The cutoff indices (for determination, see Example 2) of the two methods are listed in the following table and additionally compared with the most sensitive anti-HIV assay known to date.

Seroconversion panel (from BBI)[xx]		MAb <p24>A	MAb <p24>B	RT	gp41 peptide 1	gp41 peptide 2	Most sensitive <HIV> assay	Enzymun Kombi
Q	1st sampling	0.0	0.0	0.0	0.0	0.3	negative	0.30
	2nd sampling	24.4	48	0.0	0.0	0.0	negative	0.66
	3rd sampling	246	435	0.0	0.0	0.0	negative	3.47
	4th sampling	nd	nd	nd	nd	nd	positive	2.30
W	6th sampling	0.1	0.0	0.1	0.0	0.1	negative	0.30
	7th sampling	0.5	1.1	0.0	0.0	0.1	negative	0.32
	8th sampling	5.8	14.1	0.1	0.0	0.1	negative	0.41
	9th sampling	529	806	0.0	0.0	0.0	positive	10.1
Z	2nd sampling	0.0	0.0	0.0	0.0	0.0	negative	0.31
	3rd sampling	20	25.5	0.1	0.0	0.1	negative	0.61
	4th sampling	226	262	0.0	0.0	0.0	negative	2.96
	5th sampling	0.9	1.1	3.7	82.6	277	positive	18.6
AD	2nd sampling	0.0	0.0	0.0	0.0	0.1	negative	0.30
	3rd sampling	2.5	6.4	0.1	0.0	0.1	negative	0.33
	4th sampling	96.8	200	0.0	0.0	0.0	negative	1.5
	5th sampling	663	832	0.0	0.0	0.0	positive	> 23.3
	6th sampling	549	709	0.6	2.7	2.8	positive	> 23.3
AF	3th sampling	0.1	0.6	0.2	0.0	0.2	negative	0.31
	4th sampling	0.4	1.7	0.0	0.0	0.02	negative	0.30
	5th sampling	2.1	4.6	0.02	0.0	0.0	negative	0.34
	6th sampling	31.2	61.6	0.1	2.9	204	positive	18.0

xx BBI Boston Biomedica Inc.

This comparison shows that combined determination of p24 antigen and HIV antibodies can be markedly improved by the microspot assay format by comparison with conventional methods. Thus, the combined microspot assay is many times more sensitive than the Enzymun® combination assay in which all the antigens and antibodies are present in mixed form. There was earlier positive detection with the tested five seroconversion panels for nine samplings by comparison with the most sensitive antibody assay and even for six samplings by comparison with the Enzymun® combination assay.

4. Combined determination of p24 antigen and anti-p24 antibodies by the back-titration principle

For combined determination of the p24 antigen and of antibodies against p24 in the same array system, a p24 antigen assay was carried out in sandwich format and an anti-p24 antibody assay was carried out in back-titration format.

Arrays with the following p24-specific reagents were produced in each case on single spots (see Example 1):

(a) panel with p24 antigen and anti-p24 antibody assay:

(i) p24 antigen assay:

assay area 1: monoclonal anti-p24 antibody A Fab' fragment, biotinylated (100 µg/ml)

assay area 2: monoclonal anti-p24 antibody B Fab' fragment, biotinylated (100 µg/ml)

(ii) anti-p24 assay in back-titration design:

assay area 3: biotinylated p24 antigen

(0.3 µg/ml)

(b) comparative panel with anti-p24 antibody assay in bridge design:

- biotinylated p24 antigen (14 µg/ml)

When carrying out the assay, 30 µl of sample diluted in the ratio 1:1 with sample buffer were pipetted into each panel and incubated at an incubation temperature of 37°C with shaking for 45 min. Aspiration of the sample and washing with washing buffer were followed by addition of 30 µl of reagent solution 1 which comprises a mixture of all digoxigenin-labeled HIV antigens and HIV antibodies, and incubation at 37°C with shaking for 10 min. The following p24-specific reagents were employed:

(a) panel with p24 antigen and anti-p24 antibody assay:

- monoclonal anti-p24 antibody D $F(ab')_2$ fragment, digoxigenylated (500 ng/ml)
- monoclonal anti-p24 antibody E $F(ab')_2$ fragment, digoxigenylated (500 ng/ml)

(b) comparative panel with anti-p24 antibody assay in bridge design:

- digoxigenylated p24 antigen (30 ng/ml)

Aspiration of reagent solution 1 and washing with washing buffer were followed by addition of 30 µl of reagent solution 2 with detection reagent (see Example 1). This detection reagent was incubated at 37°C with shaking for 5 min and then aspirated, washed and dried.

The assay field was irradiated with an He-Ne laser with a wavelength of 633 nm, and the fluorescence was measured at a wavelength of 670 nm with a confocal laser scanner.

11 samples negative for HIV and 19 positive for HIV were measured comparatively with the two panels: the cutoff indices (COI) for the two assay formats are indicated in the table below.

Sample number	**COI <p24>** back titration*	**COI <p24>** bridge format**
Negative control	2412 Cts	93 Cts
Positive control	1276 Cts	24651 Cts
Negative sample 145	1.39	0.3
Negative sample 196	1.54	0.2
Negative sample 122	1.43	0.1
Negative sample 160	1.41	0.2
Negative sample 141	1.28	0.2
Negative sample 168	1.58	0.2
Negative sample 222	1.38	0.2
Negative sample 280	1.42	0.2
Negative sample 232	1.32	0.2
Negative sample 201	1.54	0.3
Negative sample 211	1.33	0.2
Positive sample 154	0.31	534
Positive sample 132	0.47	537
Positive sample 130	0.42	547
Positive sample 138	0.46	473
Positive sample 163	0.47	591
Positive sample 176	0.39	505
Positive sample 204	0.39	531
Positive sample 167	0.39	588
Positive sample 221	0.79	351
Positive sample 174	0.30	506
Positive sample 285	0.43	506
Positive sample 150	0.76	422
Positive sample 179	0.58	596
Positive sample 236	0.55	573
Positive sample 337	0.60	491
Positive sample 203	0.35	573
Positive sample 147	0.72	610
Positive sample 285	0.47	584

Positive sample 289	0.30	496

* COI = $signal_{sample}$-$signal_{background}$/0.7×$signal_{negative\ control}$; COI > 1.0 = negative

** COI = $signal_{sample}$-$signal_{background}$/2×$signal_{negative\ control}$; COI > 1.0 = positive

Correct detection with the back-titration principle was possible for both all the negative and all the positive samples. It was possible through the interaction-free combination of p24 antigen and anti-p24 antibody assays to detect seroconversion samples earlier, and additionally to increase the reliability in relation to false-negative detections.

5. Improving the assay specificity by assay area-specific cutoff calculation

In routine assays available to date, all the antigens and antibodies necessary for the determination are mixed, and a cutoff limit optimal for this mixture is established. This is determined by the nonspecific binding of the "worst" starting material. It is possible in contrast by the microspot technology of the invention to carry out an assay area-specific cutoff calculation which is specific for each starting material.

With identical calculation of the cutoff index (COI = $signal_{sample}$-$background_{sample}$/2×$background_{negative\ control}$) of the individual assay areas to achieve the following specificity with the HIV combination assay (Example 3) in 1264 samples:

- p24 antigen: 100%
- anti-HIV antibody assay: 99.52% (six false-positive determinations)

Since the false-positive determinations occurred exclusively in the two assay areas for gp41 peptide 2

and reverse transcriptase, the cutoff limits for these assay areas were increased to the following limits:

gp41 peptide 2: $COI = signal_{sample} - background_{sample} / 5 \times background_{negative\ control}$

RT: $COI = signal_{sample} - background_{sample} / 3 \times background_{negative\ control}$

It was possible in this way to improve the specificity of the HIV assay from 99.52% to 99.92% (now only a single false-positive determination). The sensitivity of the assay also remained uninfluenced because the cutoff index of the sensitive p24 antigen assay was unchanged. It is thus possible to achieve a marked improvement in specificity with an unaltered high sensitivity.

Claims

1. Method for detecting an analyte in a sample, comprising the steps:
   (a) providing a solid phase which includes a nonporous support and at least two spatially separate assay areas, where the assay areas each comprise different, immobilized analyte-specific receptors,
   (b) contacting the sample with the solid phase and at least one free analyte-specific receptor which carries a signal-emitting group or is capable of binding to a signal-emitting group, and
   (c) detecting the presence or/and the amount of the analyte by determining the signal-emitting group on the assay areas.

2. Method according to Claim 1, characterized in that the analyte to be detected represents a homogeneous or heterogeneous population.

3. Method according to Claim 1 or 2, characterized in that the analyte represents a heterogeneous antibody population, an antigen mixture or a mixture of, where appropriate, different antigens and antibodies.

4. Method according to any of the preceding claims, characterized in that the assay areas have a diameter of from 0.01 to 1 mm.

5. Method according to any of the preceding claims, characterized in that the solid phase is produced by separate, direct specific application of the different analyte-specific receptors onto the spatially separate assay areas.

6. Method according to any of the preceding claims,

characterized in that the coating on the assay areas is formed in each case from a single molecule type capable of binding.

7. Method according to any of the preceding claims, characterized in that a solid phase which additionally includes at least one control area which does not contain any analyte-specific receptor is used.

8. Method according to any of the preceding claims, characterized in that a universal detection reagent, in particular labeled latex particles, are used for detecting complexes formed from the analyte and reagents capable of binding therewith.

9. Solid phase for detecting an analyte in a sample, characterized in that it includes a nonporous support and at least two spatially separate assay areas, where the assay areas each comprise different reagents which specifically bind the analyte to be determined.

10. Solid phase according to Claim 9, characterized in that the assay areas each comprise different reagents which bind to different epitopes or/and subtypes of the analyte or/and to different analyte types.

11. Solid phase according to Claim 9 or 10, characterized in that the nonporous support is formed from polystyrene.

12. Solid phase according to any of Claims 9 to 11, characterized in that the assay areas have a diameter of from 0.01 to 1 mm.

13. Use of a solid phase according to any of Claims 9 to 12 in an immunoassay.

14. Assay kit for detecting an analyte in a sample including a solid phase according to any of Claims 9 to 12, and labeled detection reagents.

15. Assay kit according to Claim 14, characterized in that it comprises labeled latex particles as universal detection reagent.

16. Method for the simultaneous determination of an antigen and of an antibody specifically directed against this antigen in a sample, including the steps:

(a) providing a solid phase on which an immobilized receptor capable of binding with the antigen to be determined is applied in a first assay area, and an immobilized receptor capable of binding with the antibody to be determined is applied in a second assay area spatially separate therefrom,

(b) contacting the sample with the solid phase and with a free analyte-specific receptor which carries a signal-emitting group or is capable of binding with a signal-emitting group, and

(c) detecting the presence or/and the amount of the antigen and of the antibody by determining the signal-emitting group on the solid phase.

17. Method according to Claim 16, characterized in that the detection of the antigen is carried out using a sandwich assay.

18. Method according to either of Claims 16 or 17, characterized in that the detection of the antibody is carried out using a back-titration design.

19. Method according to Claim 16 or 17, characterized in that the detection of the antibody is carried out using a bridge design.

20. Method according to either of Claims 16 or 17, characterized in that the detection of the antibody is carried out using an indirect assay format.

21. Method according to any of Claims 16 to 20, characterized in that the coating, which is capable of binding, of the first assay area is formed from immobilized antibodies which are specific for an epitope of the antigen to be detected.

22. Method according to Claim 21, characterized in that antibodies which are specific for different subtypes of the antigen to be detected are applied in separate assay areas.

23. Method according to Claim 21 or 22, characterized in that the antibody is selected from viral antibodies, especially anti-HIV-I antibodies, anti-HIV II antibodies, anti-HBV antibodies and anti-HCV antibodies.

24. Method according to any of Claims 16 to 23, characterized in that the coating, which is capable of binding, of the second assay area is formed from antigens which are specific for the antibody to be detected.

25. Method according to Claim 24, characterized in that the antigens are selected from the group consisting of HIV-I, HIV-II, HBV and HCV.

26. Method according to any of Claims 16 to 25, characterized in that the antigen to be determined

is HIV p24 and the antibody to be determined is anti-p24.

27. Method according to any of Claims 16 to 26, characterized in that a nonporous solid phase is used.

28. Method according to any of Claims 16 to 27, characterized in that the detection is carried out using labeled antibodies which are directed against the analytes.

29. Method according to Claim 28, characterized in that the label is selected from fluorescent groups, chemiluminescent groups, radioactive labels, enzyme labels, colored labels and sol particles.

30. Method according to any of Claims 16 to 29, characterized in that the detection is carried out using a universal detection reagent, in particular labeled latex particles.

31. Method according to any of Claims 16 to 30, characterized in that the solid phase is produced by direct, separate application of the coatings capable of specific binding onto the individual assay areas.

32. Method according to any of Claims 16 to 31, characterized in that the coating on the assay areas is formed in each case from a single molecule type capable of binding.

33. Solid phase for simultaneous determination of an antigen and of an antibody specifically directed against this antigen in a sample, comprising at least a first assay area and at least a second assay area, characterized in that the first assay

area includes a coating capable of specific binding with an antigen, and the second assay area includes a coating capable of specific binding with an antibody directed against the antigen.

34. Solid phase according to Claim 33, characterized in that the coatings are homogeneous and each comprise only a single type of a reagent capable of binding.

35. Solid phase according to Claim 33 or 34, characterized in that the assay areas are applied to a nonporous support.

36. Solid phase according to Claim 35, characterized in that the nonporous support is formed from polystyrene.

37. Solid phase according to any of Claims 33 to 36, characterized in that the individual assay areas have a diameter of from 0.01 to 1 mm.

38. Use of a solid phase according to any of Claims 33 to 37 in an immunoassay for the simultaneous detection of an antigen and of an antibody specifically directed against this antigen.

39. Assay kit for simultaneous determination of an antigen and of an antibody specifically directed against this antigen, including a solid phase according to any of Claims 33 to 37, and labeled detection reagents.

40. Assay kit according to Claim 39, characterized in that it includes a universal detection reagent.

41. Method for detecting an analyte in a sample, including the steps:
(a) providing a solid phase which includes a

support and at least two spatially separate assay areas, where the assay areas each comprise different, immobilized analyte-specific receptors,

(b) contacting the sample with the solid phase and at least one free analyte-specific receptor which carries a signal-emitting group or is capable of binding to a signal-emitting group, and

(c) detecting the presence or/and the amount of the analyte by determining the signal-emitting group on the assay areas, where a signal above a predetermined assay area-specific limit is classified as positive and below a predetermined assay area-specific limit is classified as negative.

42. Method according to Claim 41, characterized in that the limits are determined in each case individually for an assay area.

43. Method according to Claim 41 or 42, characterized in that the limits are established differently for at least two assay areas.

Abstract

A method for detecting an analyte in a sample, comprising the steps:

(a) providing a solid phase which includes a nonporous support and at least two spatially separate assay areas, where the assay areas each comprise different, immobilized analyte-specific receptors,

(b) contacting the sample with the solid phase and a second analyte-specific receptor which carries a signal-emitting group or is capable of binding to a signal-emitting group, and

(c) detecting the presence or/and the amount of the analyte by determining the signal-emitting group on the solid phase,

is described.